Acute Toxicity of Pesticide Mixtures to Bluegills

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The U.S. DEPARTMENT OF AGRICULTURE (1974) has recommended at least 19 mixtures of registered pesticides for the control of various insect or other pests. In addition, two or more pesticides applied separately may occasionally contaminate an aquatic habitat. For example, one chemical may be applied to control the aquatic larval stages of insect pests, and another to control aquatic algae or rooted vegetation. Consequently, a knowledge of the toxicity of such pesticide combinations is necessary to protect fishery resources and establish safe use patterns for these chemicals.

The action of a few insecticide mixtures has been investigated in warm-blooded vertebrates and fish (FRAWLEY, et al., 1958, ROSENBERG and COON 1958, TRIOLO and COON 1966, MAYER, et al., 1970, MAYER, et al., 1972). However, other approaches that have been described for evaluating joint toxicity of chemical combinations (BLISS 1939, FINNEY 1952, SMYTH et al., 1969, SMYTH et al., 1970, MARKING and DAWSON, unpublished) have apparent shortcomings: each evaluation requires certain assumptions, statistical errors are not developed satisfactorily for toxicity indices, and rather involved algebraic manipulations are necessary for the development of reference points and linearity. Instead of attempting to develop a satisfactory mathematical model applicable to any combination of chemicals, I empirically evaluated the acute toxicity of 29 two-chemical combinations to bluegills, Lepomis macrochirus, to attempt to discern possible trends in less than additive, additive, or greater than additive toxicity. Although indices expressing these trends are preliminary, I hope to stimulate the development of better methods for evaluating toxicity of combinations of chemicals and to call attention to mixtures that may be more toxic or less toxic to fish than the individual pesticides alone.

Materials and Methods

The common name, chemical name, and purity of the pesticides used are given in Table 1. Bluegills used in the toxicity tests were obtained from several National Fish Hatcheries and ranged in size from 0.8 to 1.2 g. The test fish were maintained in laboratory facilities (BRAUHN and SCHOETTGER in press) for at least 10 days prior to testing. Mortality in the test population was

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Table 1. Pesticides tested against bluegills.

Common or trade name	Chemical name	Active ingredient (%)
Baytex®	0,0-dimethyl 0- [4-(methylthio)-m-tolyl] phosphorothioate	47.5
BHC	1,2,3,4,5,6-hexachlorocyclohexane	41.5
Sevin®	1-napthyl N-methylcarbamate	99
Chlordane (technical)	octachloro-4,7-methanetetrahydroindane isomers	100
Copper sulfate	Cupric sulfate (pentachydrate)	64
DDT	2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane	99.9
Diazinon	0,0-diethyl 0-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate	91.8
Dichlorvos (DDVP)	0,0-dimethyl 0-2,2-dichlorovinyl phosphate	93
Dieldrin	not less than 85% of the 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octachydro-1,4-endo-exo-5,8 dimethano-napthalene	85
Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodiozathiepin-3-oxide	96
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonapthalene	97
EPN	0-ethyl 0-p-nitrophenyl-phosphorothioate	100
Malathion	0,0-dimethyl S-(1,2-dicarbethoxy) ethyl phosphoro dithioate	95
Methoxychlor	2,2-bis (p-methoxyphenyl)-1,1,1-trichloroethane	89.5
Methyl parathion	0,0-dimethyl 0-p-nitrophenyl phosphorothioate	80
Parathion	0,0-diethyl 0-p-nitrophenyl phosphorothioate	98.7
Perthane®	2,2 bis (p-ethylphenyl)-1,1-dichloroethane	95
Toxaphene	chlorinated camphene containing 67-69% chlorine	100
Zectran®	4-dimethylamino-3,5-xylyl N-methylcarbamate	95

less than 10% during this period and the fish were judged to be in good physical condition. Toxicity tests were conducted in 19-liter glass vessels held in constant temperature water baths (18 ± 0.5 C). The test diluent consisted of 15 liters of deionized water of at least 1 million ohms resistivity, to which were added the following chemicals (mg/l) potassium chloride, 2; calcium sulfate, 30; magnesium sulfate, 30; and sodium bicarbonate 48. The pH of the diluent was 7.1 and the methyl orange alkalinity 35 ppm. Toxicity tests were conducted under static conditions without aeration and with a single introduction of the toxicant(s). Fish were conditioned to the test water and temperature for at least 24 hr before testing. Test concentrations, as active ingredient, were prepared by adding appropriate amounts of pesticides, dissolved in acetone, to 15 liters of the diluent. In the tests of combinations, each chemical was introduced separately but simultaneously. The mass/volume ratio never exceeded 1.0 g of fish per liter of water. The control fish were exposed to the highest concentration of the solvent to which fish in the test solutions were exposed. Ten fish were used in each test vessel; at least two, and usually three, replicates were run for each concentration.

Table 2. Acute toxicity of combinations of pesticides to bluegills.

Combination[a] and concentration range (ug/l) A	B	Exposure (hr)	Mortality (E%) AB/A+B =	R[b]	Combined action[c]
Malathion (52-92)	Parathion (35-60)	24	270/23	11.7	GA[d]
Baytex (60-80)	Malathion (20-35)	48	153/35	4.37	GA
Parathion (4-80)	Methoxychlor (21-32)	48	163/49	3.31	GA
DDT (4.5-5.0)	BHC (1.5-60.0)	72	205/82	2.50	GA[e]
Sevin (3200-3800)	Copper Sulfate (870-1500)	72	244/110	2.21	GA
Endrin (0.3-0.6)	Methyl Parathion (3700-5400)	72	228/105	2.17	GA
Sevin (140-4500)	Malathion (70-80)	72	240/118	2.03	GA[e]
EPN (40-70)	Malathion (35-55)	48	166/86	1.93	GA
Malathion (24-32)	Perthane (12-30)	48	190/105	1.82	GA[e]
Parathion (70-120)	Copper Sulfate (870-1500)	48	176/110	1.60	GA
Diazinon (100-140)	Parathion (70-90)	48	202/127	1.59	GA
Parathion (70-90)	Endosulfan (0.8-1.5)	48	116/87	1.34	A[e]
Sevin (3700-5400)	Methyl Parathion (3700-5400)	72	139/106	1.31	A[e]
DDT (4.5-5.0)	Malathion (5-103)	72	100/76	1.31	A
Malathion (5-9)	Toxaphene (15-27)	48	176/156	1.14	A[e]
DDT (4.5-5.0)	Dieldrin (5.9-6.6)	96	80/75	1.06	A
DDT (4.0-5.0)	Toxaphene (7.6-8.5)	96	275/263	1.05	A[e]
Parathion (70-82)	Chlordane (55-65)	48	96/97	0.99	A
Parathion (70-82)	Toxaphene (5.5-7.0)	48	240/248	0.97	A[d]
DDT (4.2-5.0)	Parathion (0.6-65)	48	65/68	0.96	A[e]
DDT (4.5-5.0)	Methyl Parathion (4940-5410)	72	135/141	0.95	A
DDT (4.5-5.0)	DDVP (67-80)	72	140/161	0.87	A
DDT (4.5-5.0)	Endrin (0.5-0.6)	96	170/200	0.85	A
DDT (4.5-5.0)	Zectran (8650-9630)	96	70/85	0.82	A
DDT (4.5-5.0)	Sevin (2720-3600)	72	60/76	0.79	A
Sevin (3700-4500)	Methoxychlor (20-26)	72	143/182	0.79	A
Endosulfan (1.2-1.6)	Methyl Parathion (0.9-1.2)	72	144/188	0.77	A[d]
Sevin (700-5200)	Parathion (70-82)	72	156/206	0.76	A[d]
Malathion (90-120)	Copper Sulfate (900-1000)	72	40/148	0.28	LA

[a]Concentration tested as active ingredient.

[b]Ratio (R) of the sum percentage mortality produced by the various concentration of AB to the sum of that produced by all levels of A plus that produced by all levels of B.

[c]GA = greater than additive; A=additive, LA = less than additive.

[d]This combination recommended for control of insect pests by the U.S. Department of Agriculture.

[e]Combinations recommended by Agriculture Handbook 331 (1967); dropped in 1974 revision.

Toxicity tests were simultaneously conducted by using concentration A_1, A_2 and A_3 of chemical A; B_1, B_2 and B_3 of chemical B; and A_1B_1, A_2B_2 and A_3B_3 of the combinations. On the basis of preliminary tests, I selected concentrations of the individual chemicals that would produce less than 40% mortality in 72 hours. Combinations currently recommended for the control of insect pests were also tested in the same ratio of A/B as that recommended by the U.S. Department of Agriculture. Mortality was observed at 24-hr intervals. If the actions of chemicals A and B were simply additive for a given exposure, then the ratio of the sum of the mortalities (percentage) produced at the various concentrations of AB to that produced by all levels of A plus that produced by all levels of B was approximately 1.0. If combinations yielded a ratio greater than 1.5, I arbitrarily considered the joint toxicity that resulted from this interaction to be greater than additive; if the mortality ratio (AB/A + B) was less than 0.5, I considered the joint toxicity to be less than additive. The ranges above and below unity (additive) probably were not linearly symmetrical, but for the preliminary estimates of trends reported herein, the ranges selected should suffice.

Results and Discussion

Eleven combinations of chemicals tested had greater than additive toxicity to bluegills (Table 2). Six of these combinations were later tested with rainbow trout (Salmo gairdneri) to compare species susceptibility and the results were closely similar to those obtained with bluegills (Unpublished data, Fish-Pesticide Research Laboratory). Seventeen combinations had no more than what appears to be additive toxicity, and one-- malathion and copper sulfate -- had less than additive toxicity.

The results of the toxicity tests with DDT, one of the most widely distributed and persistent synthetic chemicals in aquatic ecosystems, were encouraging in that DDT did not have greater than additive toxicity with any of the pesticides tested except BHC. On the other hand, three observations were disquieting: First, and most important, was that greater than additive toxicity between commonly used pesticides was observed in more than one-third of the combinations tested. Second, many organophosphorus insecticides being proposed as alternatives to the more persistent pesticides were particularly active when combined with other chemicals. Specifically, malathion, a compound frequently applied directly to aquatic environments, had greater than additive toxicity with more than half of the chemicals with which it was combined. Third, several combinations currently recommended by the U.S. Department of Agriculture had greater than additive toxicity to bluegills. Although none of these particular combinations are recommended for use in aquatic environments by the Department, safety precautions are warranted because compounds such as malathion and sevin may be used independently in or near aquatic sites.

The results of this study indicate that further research on the interaction of pesticides in aquatic environments is urgently needed to protect fishery resources. In view of the difficulty of developing sufficient empirical data, a primary need is the development of a valid toxicological approach to evaluating the toxicity of combinations of chemicals.

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